# Intermicrobial interactions and sustainable biological control of bacterial diseases of plants

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Microbial Biocontrol of Arthropods, Weeds, and Plant Pathogens: Risks, Benefits and Challenges
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# Features of bacterial diseases of plants that make them amenable to biological control

- Bacterial plant pathogens colonize surfaces of plants.
- Bacteria invade tissues though natural openings (e.g. stomates, hydrathodes, and nectarthodes) or wounds.
- Reducing surface populations of pathogens or protecting wounds and natural openings interrupts the disease process.
- Few chemical control methods are available or effective, so growers are interested in biological control.

# Two classic bacterial diseases

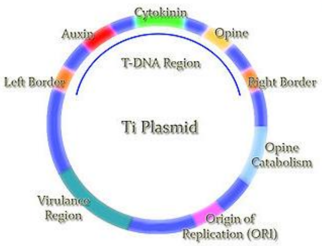
**Crown gall**
***Agrobacterium tumefaciens***

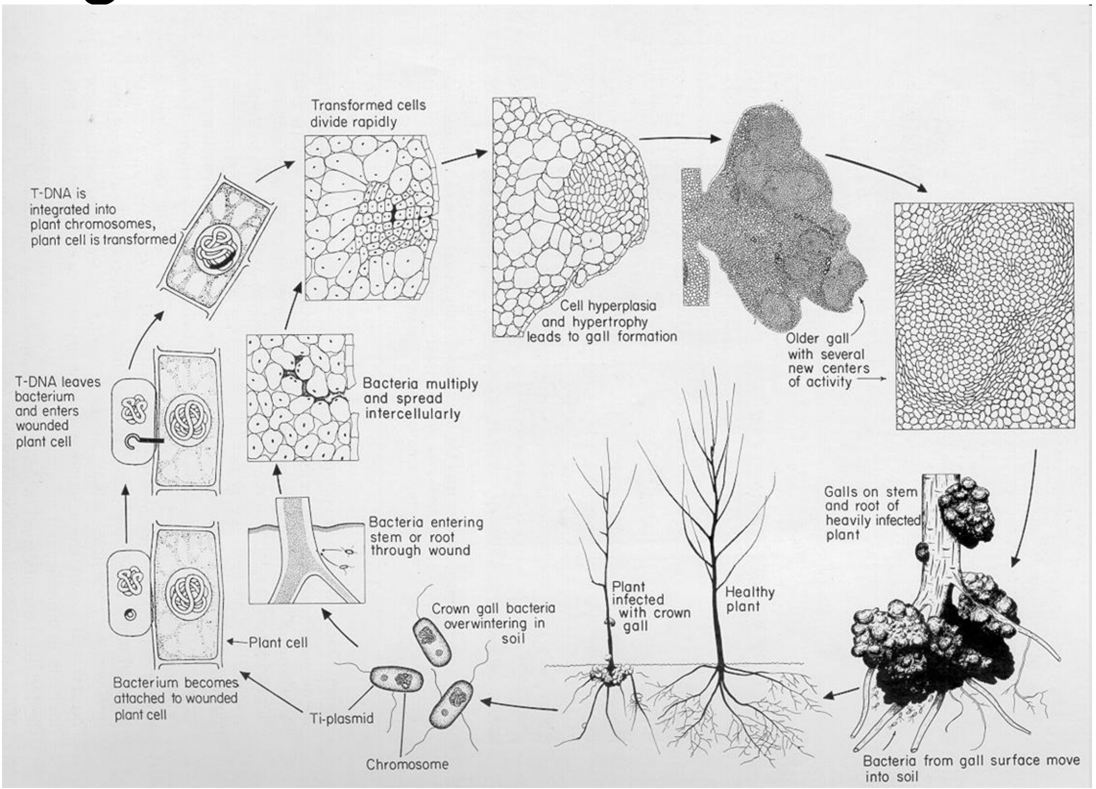
www.apsnet.org

**Fire Blight**
***Erwinia amylovora***

# Crown gall

## *Agrobacterium tumefaciens*




Historically a severe disease in plant nurseries. Infected plants must be destroyed, resulting in losses of ~80% of plants.

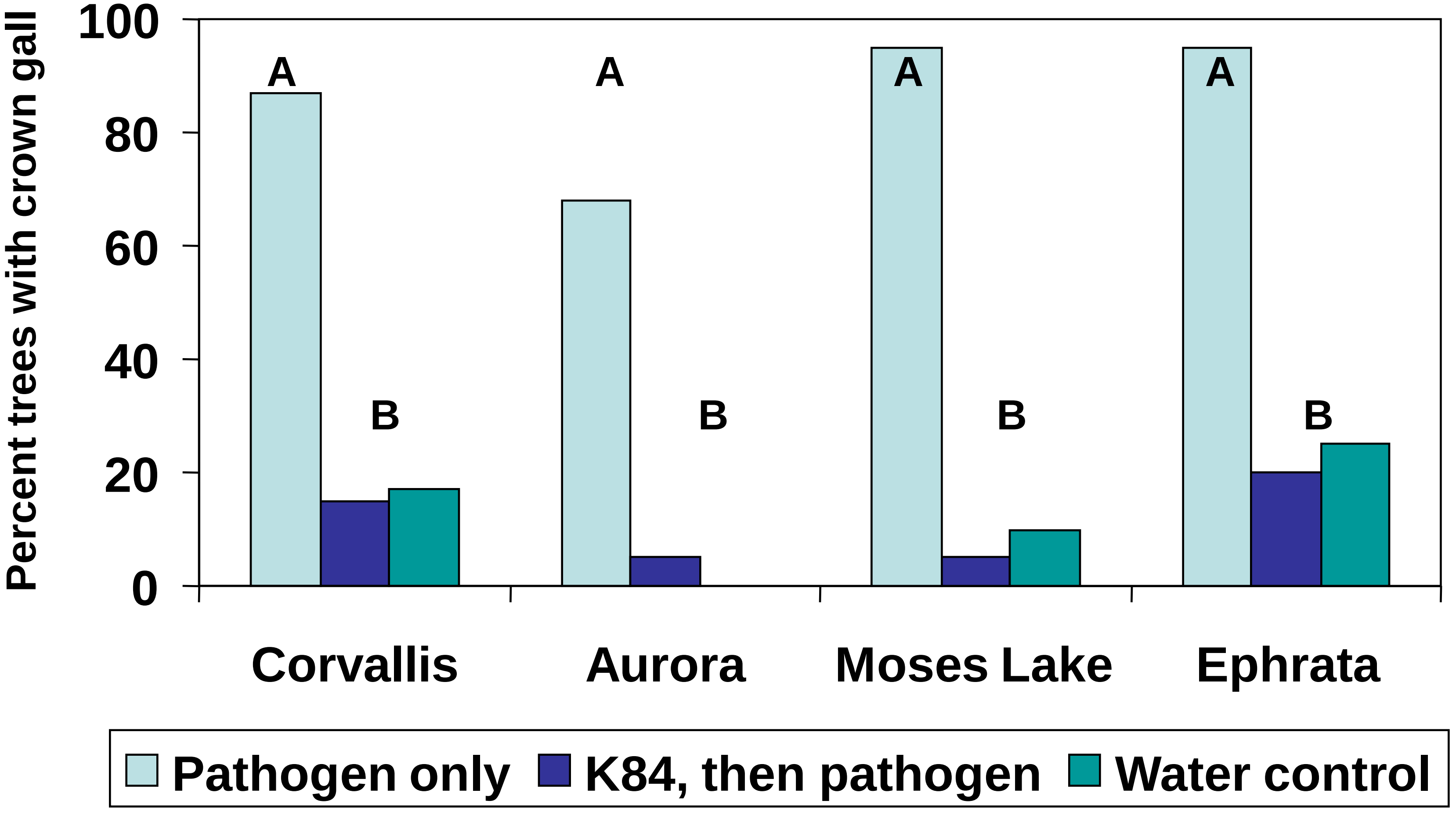
•Disease controlled by sanitation and biological control.

Disease cycle from Agrios, pTi representation from wikipedia

# *Agrobacterium radiobacter* strain K84

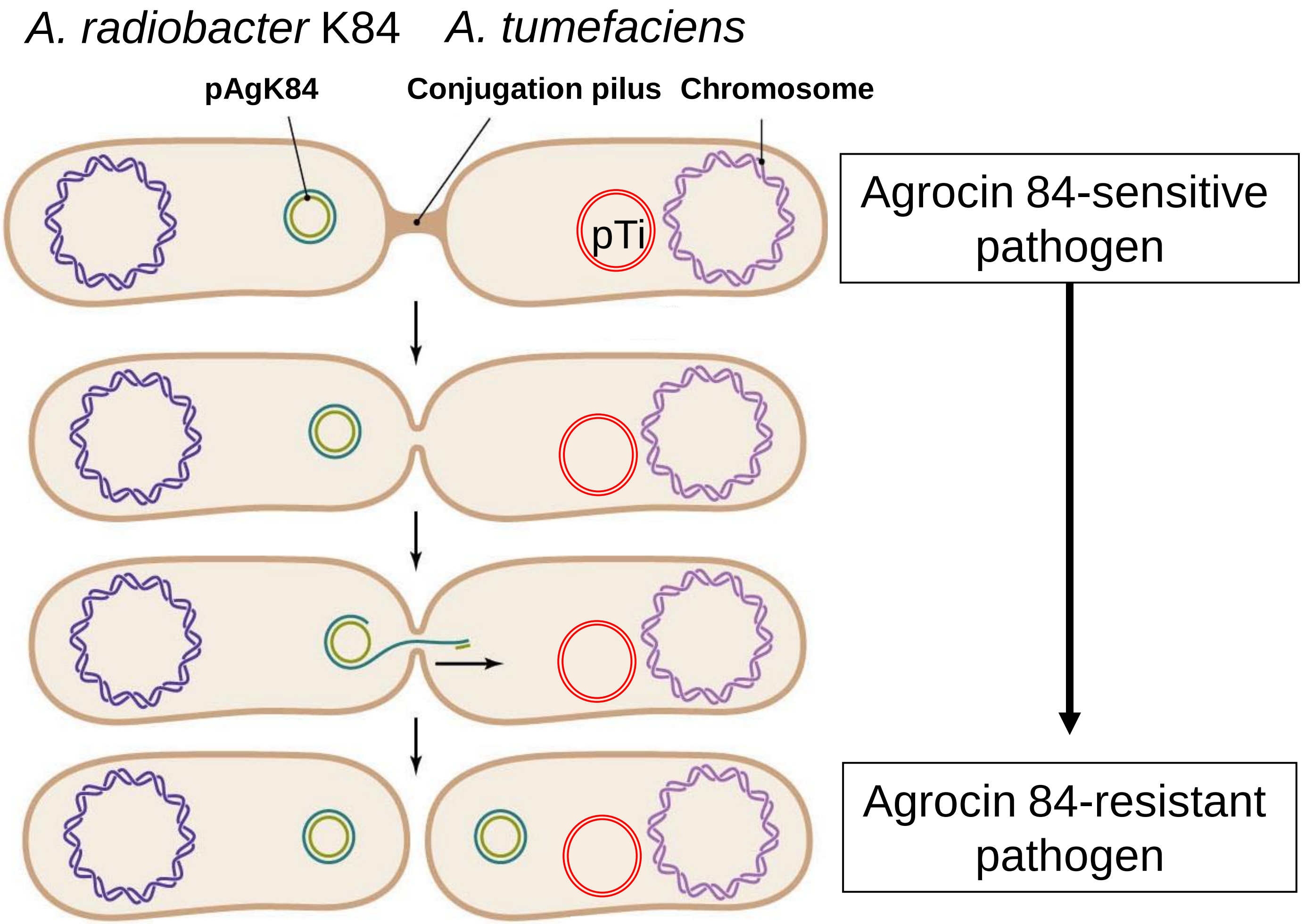
- Discovered in Australia.
- Registered and commercialized in US in 1970s
- Used primarily in nurseries
- Applied by dipping plants in ~$10^8$ CFU/ml suspension prior to planting
- K84 colonizes wounds, occupies binding sites for the pathogen, and produces an antibiotic Agrocin 84

# Efficacy of *Agrobacterium radiobacter* strain K84 on cherry rootstock



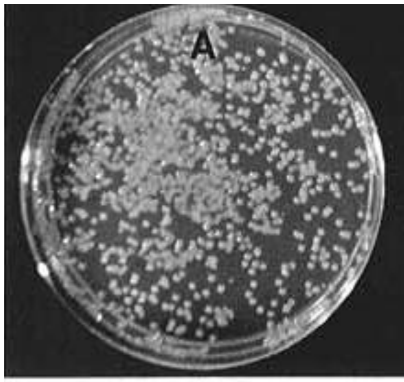
# Concern about sustainability of biocontrol of crown gall with K84

pAg84 carries Agrocin 84 production and resistance genes and is self-transmissible.

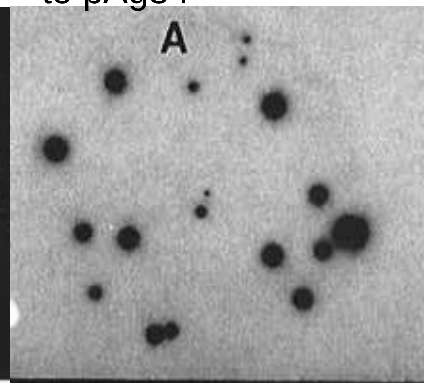


# Transfer of pAg84 to pathogenic agrobacteria in plants in fields

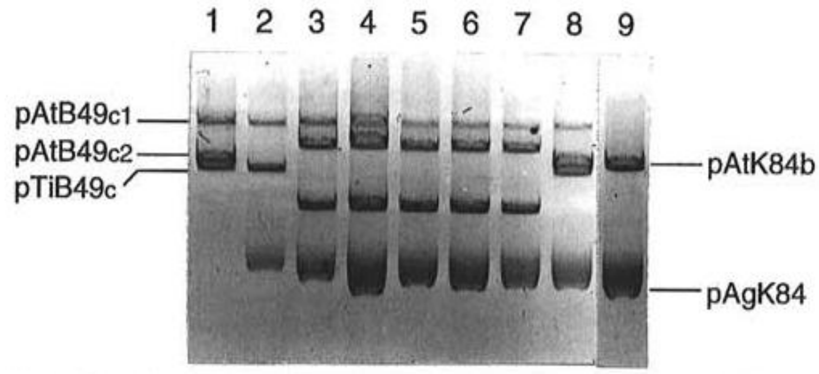
Pathogen colonies



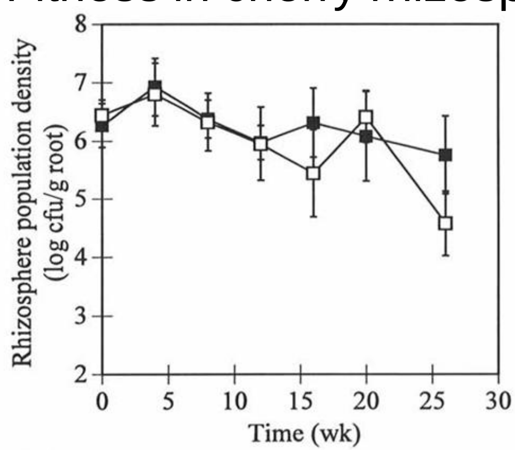

Colony hybridization to pAg84



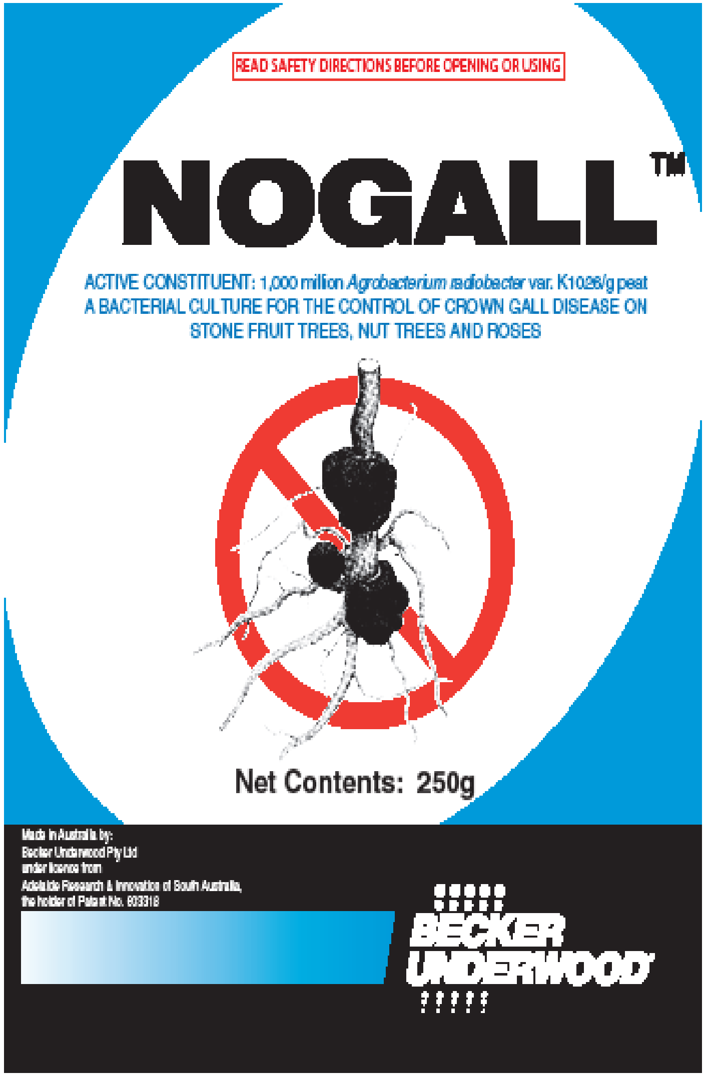

Plasmids in pathogenic field transconjugants




Fitness in cherry rhizosphere




From Stockwell et al, 1996 Phytopathology and Vicedo, B. et al 1996. Phytopathology
Reports from commercial plantings in Oregon (Lu, SF, 1994 MSc thesis, Oregon State University) and Italy (Raio, A. et al 2009 Plant Pathology)




Strain K1026, a genetically engineered derivative of K84

- Deleted *tra* gene in pAg84

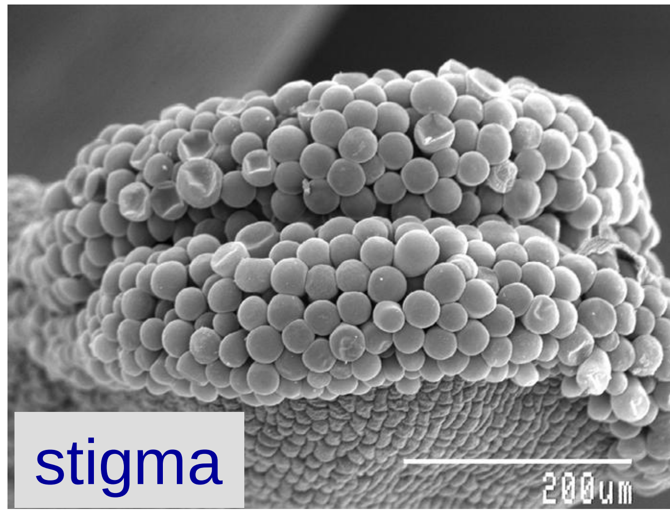
K1026 produces Agrocin 84 and controls crown gall as well as the wild type, but can no longer transfer pAg84 to the pathogen

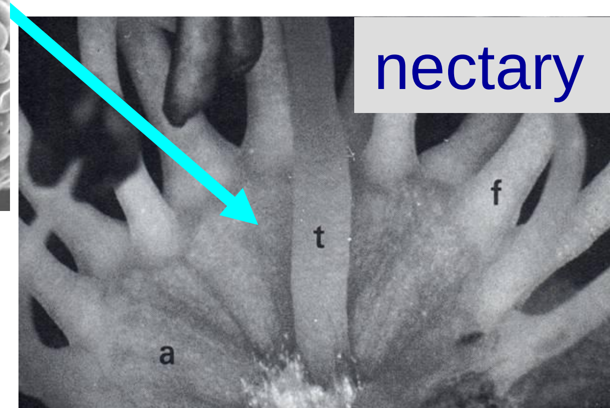
Registered and commercialized in US in 1999

# Fire blight: bacterial disease of pear and apple caused by *Erwinia amylovora*

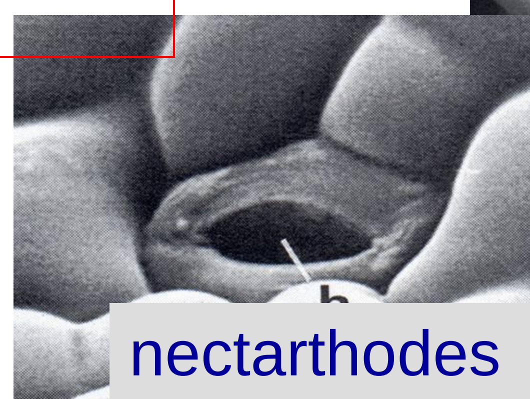
Fire blight epidemics occur sporadically, resulting in losses of tens of millions of dollars to growers.

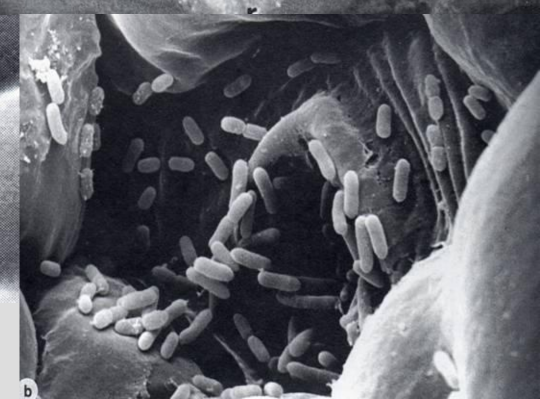
*Erwinia amylovora* grows as an epiphyte on floral stigmas to populations of $10^6$ to $10^7$ cells per flower then moves to infection sites on the nectary




**Prevent disease by suppressing epiphytic growth of pathogen on stigmas, before migration to the nectary**



Micrographs by Mark Wilson and Larry Pusey

# Biological control agents for fire blight

***Pseudomonas fluorescens* A506 (BlightBan A506, NuFarm Americas)**

- Isolated from pear in California by Steve Lindow.
- Primary mechanism: preemptive exclusion.
- Registered and commercialized for fire blight management (1996).
- Also used to reduce frost injury and russet of fruit.

***Pantoea vagans* C9-1 (BlightBan C9-1, NuFarm Americas)**

- Isolated from apple in Michigan by Carol Ishimaru.
- Mechanisms: peptide antibiotics and preemptive exclusion
- Formerly called *Erwinia herbicola* C9-1, then *Pantoea agglomerans* C9-1.
- Registered in 2006, but not yet commercialized, for control of fire blight.

# Experimental orchard trials

Spray biological control agents at $10^8$ CFU/ml on flowers during early and near full bloom

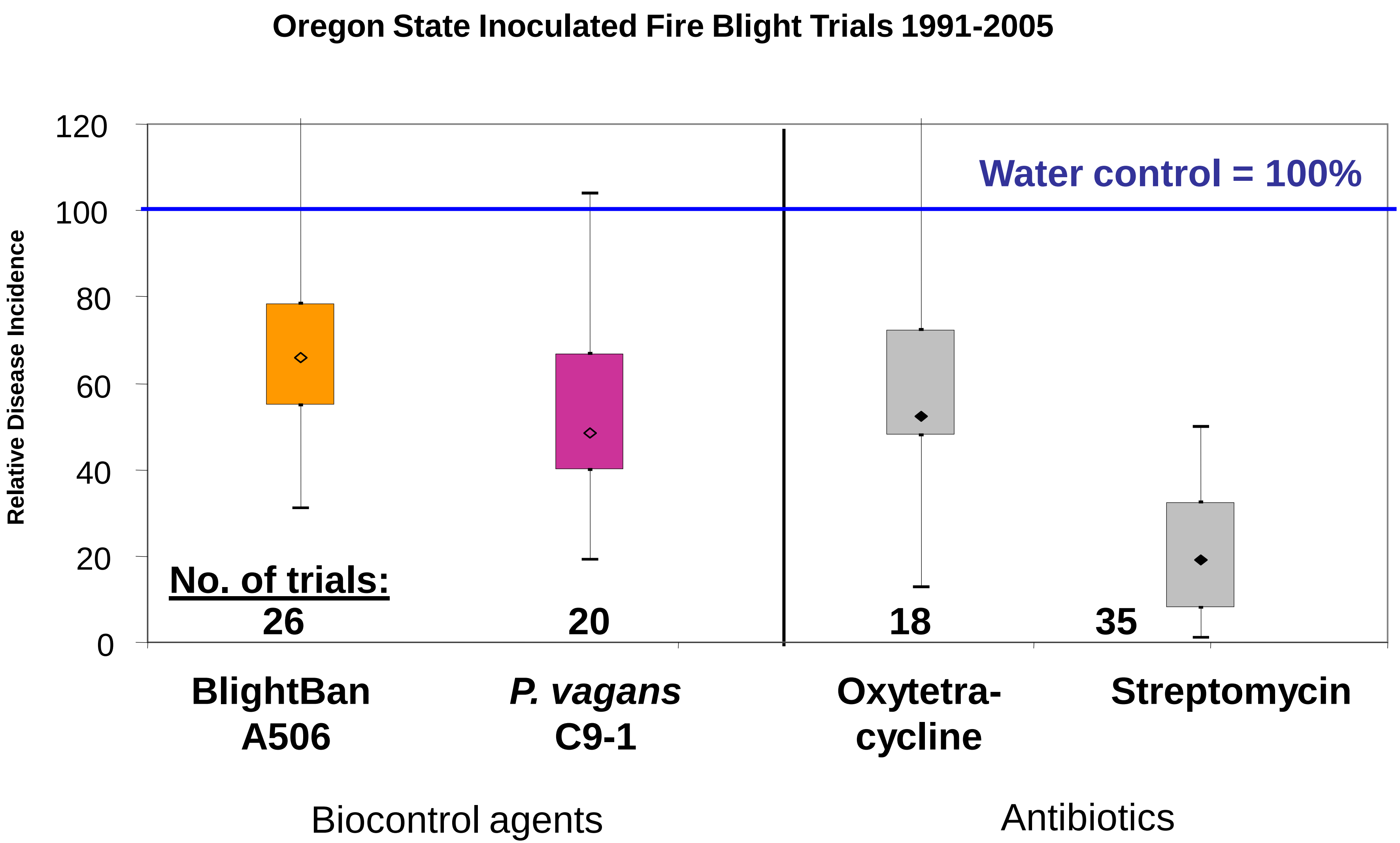
At full bloom, inoculate flowers by misting with low dose of the pathogen

Monitor bacterial populations on flowers

Count and remove infected blossom clusters

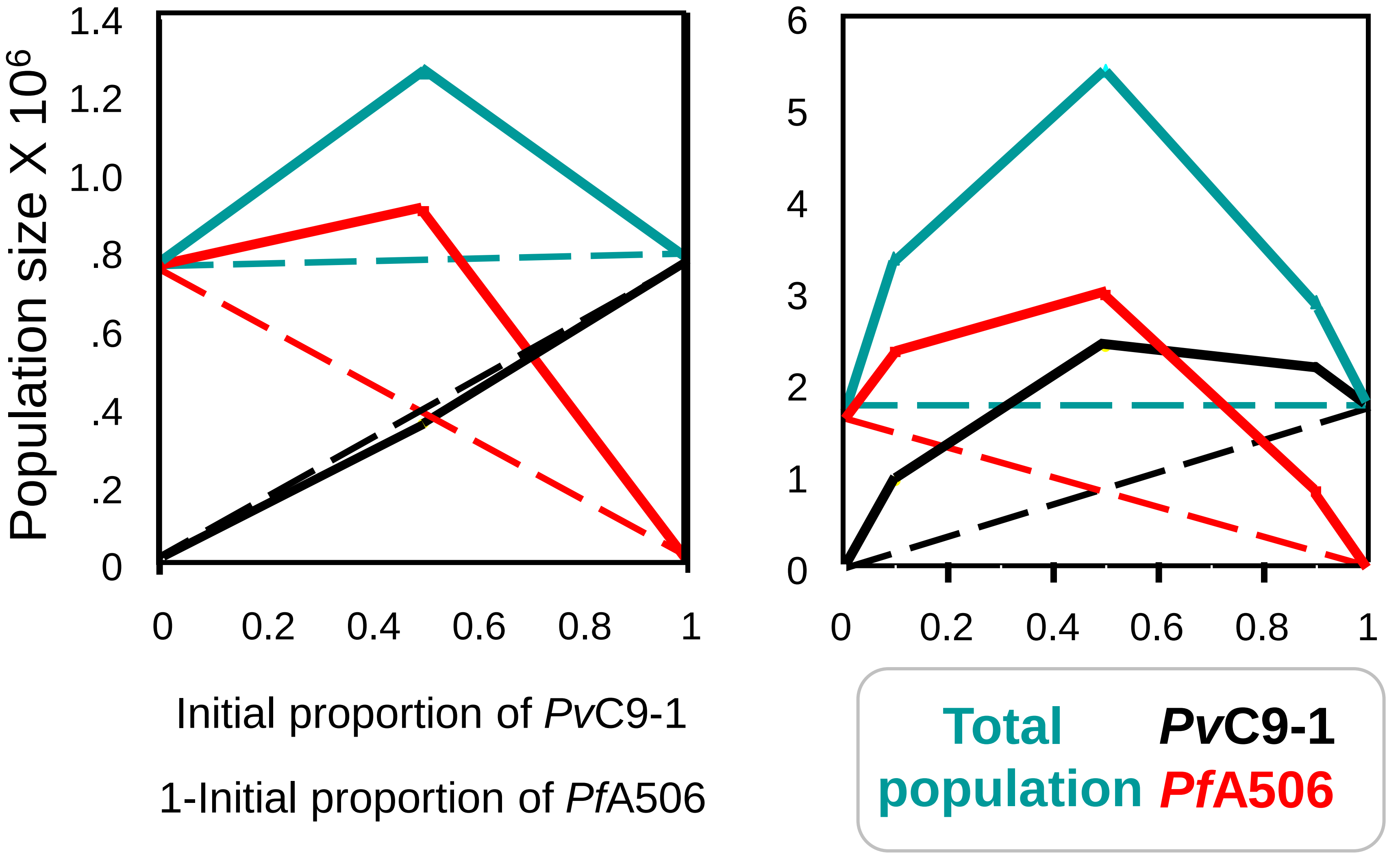
# Disease control in inoculated orchard trials

(antibiotic-sensitive isolate of *Erwinia amylovora*)

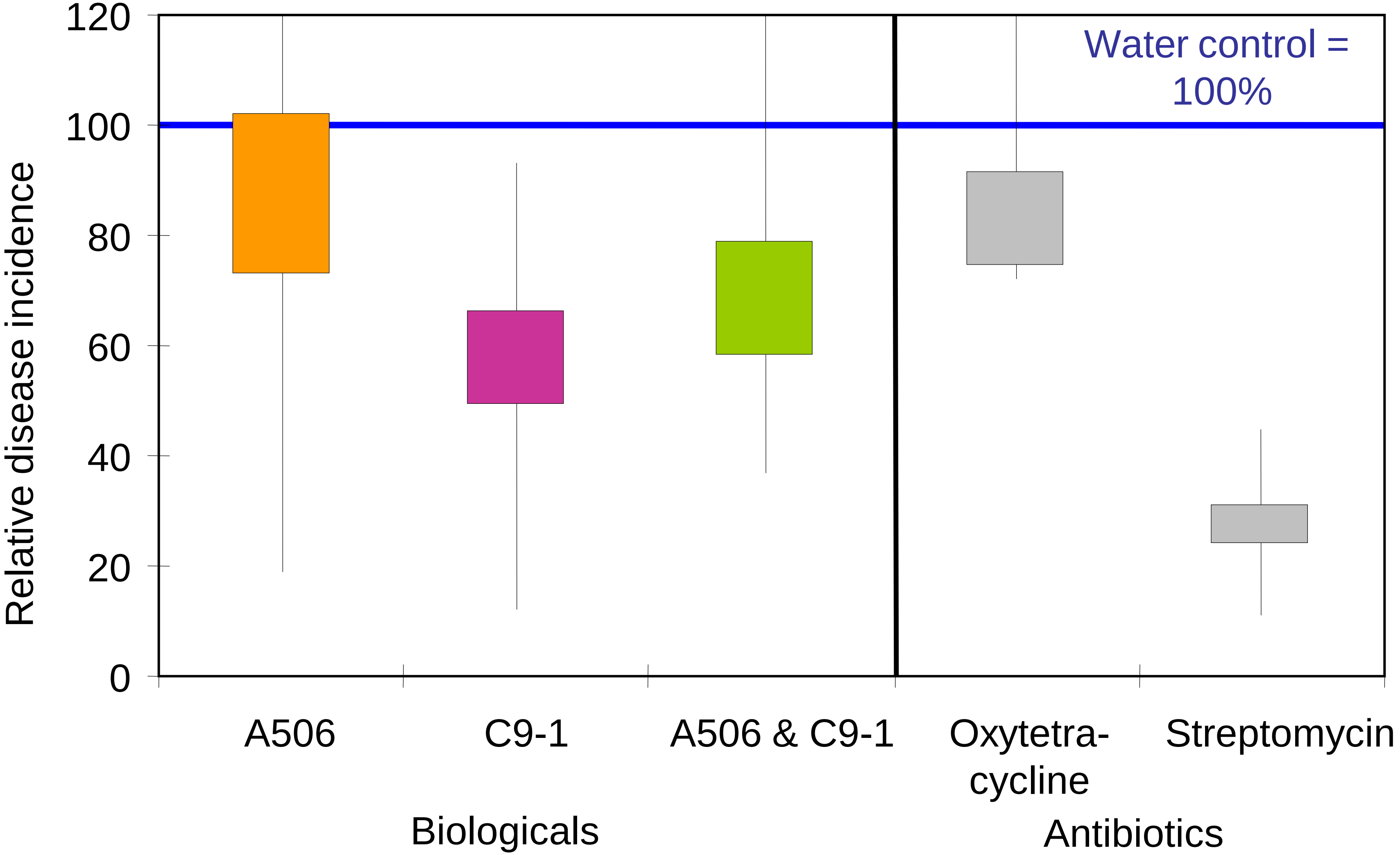


# Evaluation of intergeneric mixtures of antagonists for disease control

# Populations of *Pv*C9-1 and *Pf*A506 on flowers when applied singly and as mixtures



# Disease control with an intergeneric mixture of antagonists




Stockwell et al 2010 Phytopathology

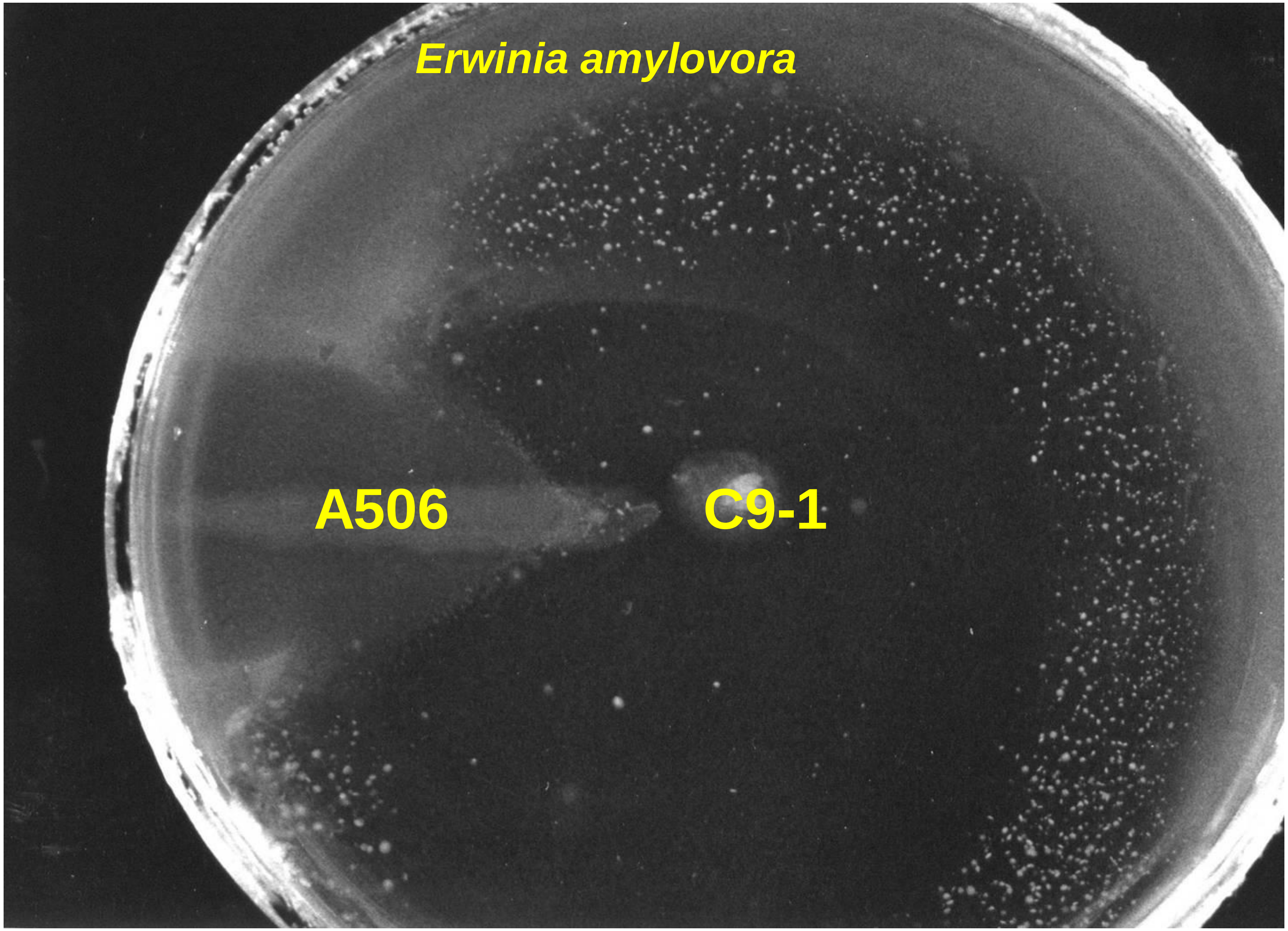
*Pantoea vagans* C9-1 and *Pseudomonas fluorescens* A506 co-colonize flowers when applied as a mixture.

Total antagonist population sizes were increased compared to single strain inoculants.

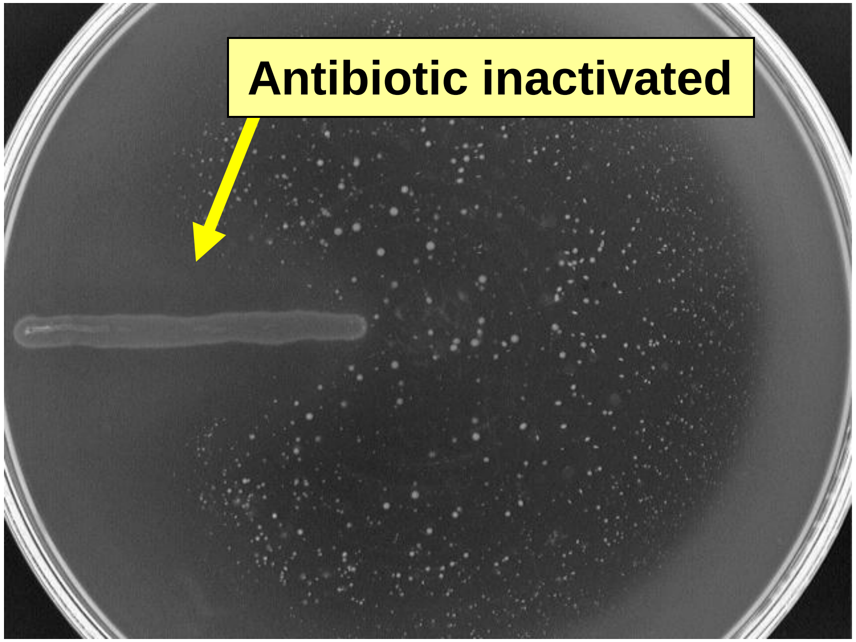
We anticipated enhanced control compared to single strains, but this did not occur.

We suspected that the interacting strains were interfering with a disease control mechanism.

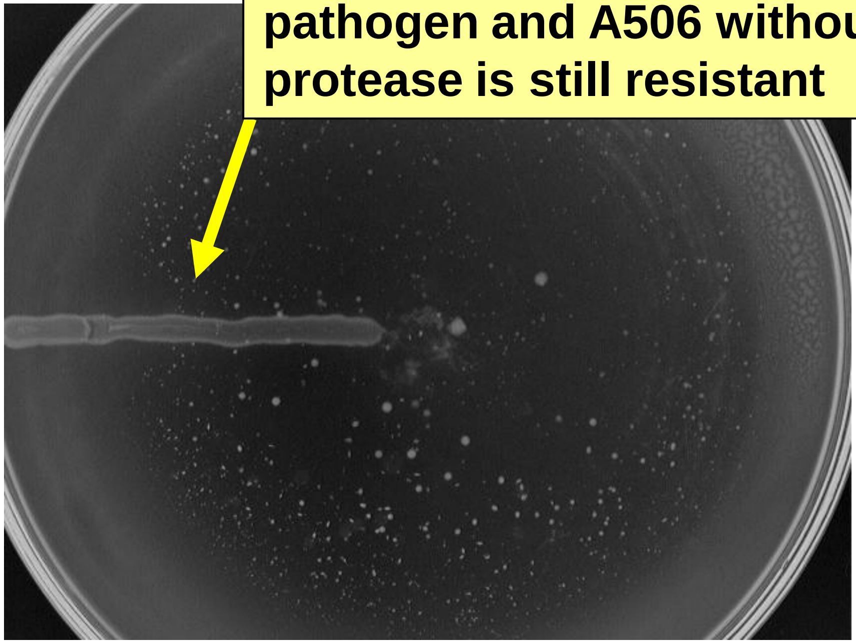
# Inhibition of *Erwinia amylovora* in culture by C9-1 and A506



# Extracellular metalloprotease (AprX) of A506 inactivates a peptide antibiotic of *Pantoea* strains



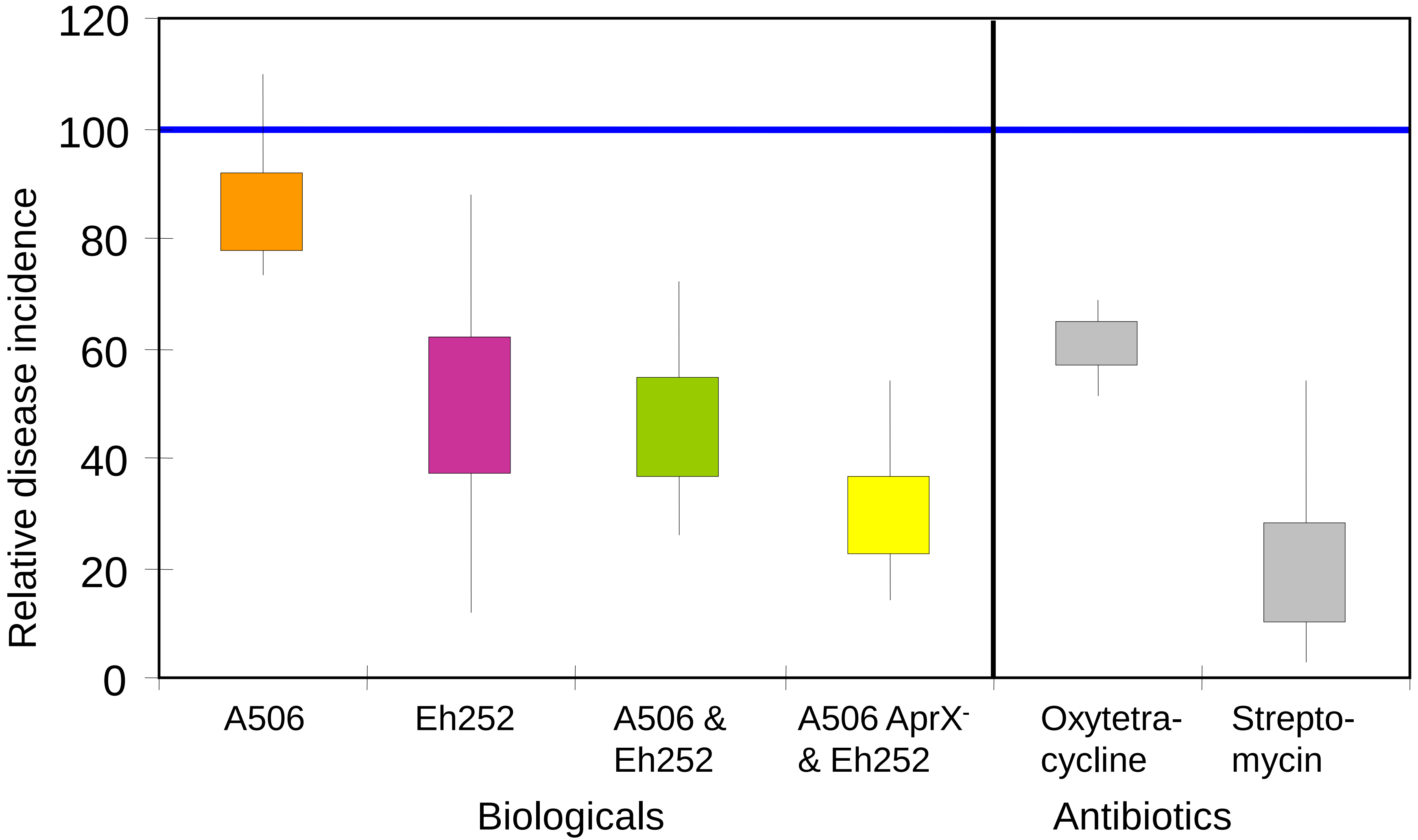

**Eh252 & A506**




**Eh252 &**
**A506 *aprX*::Tn*5***

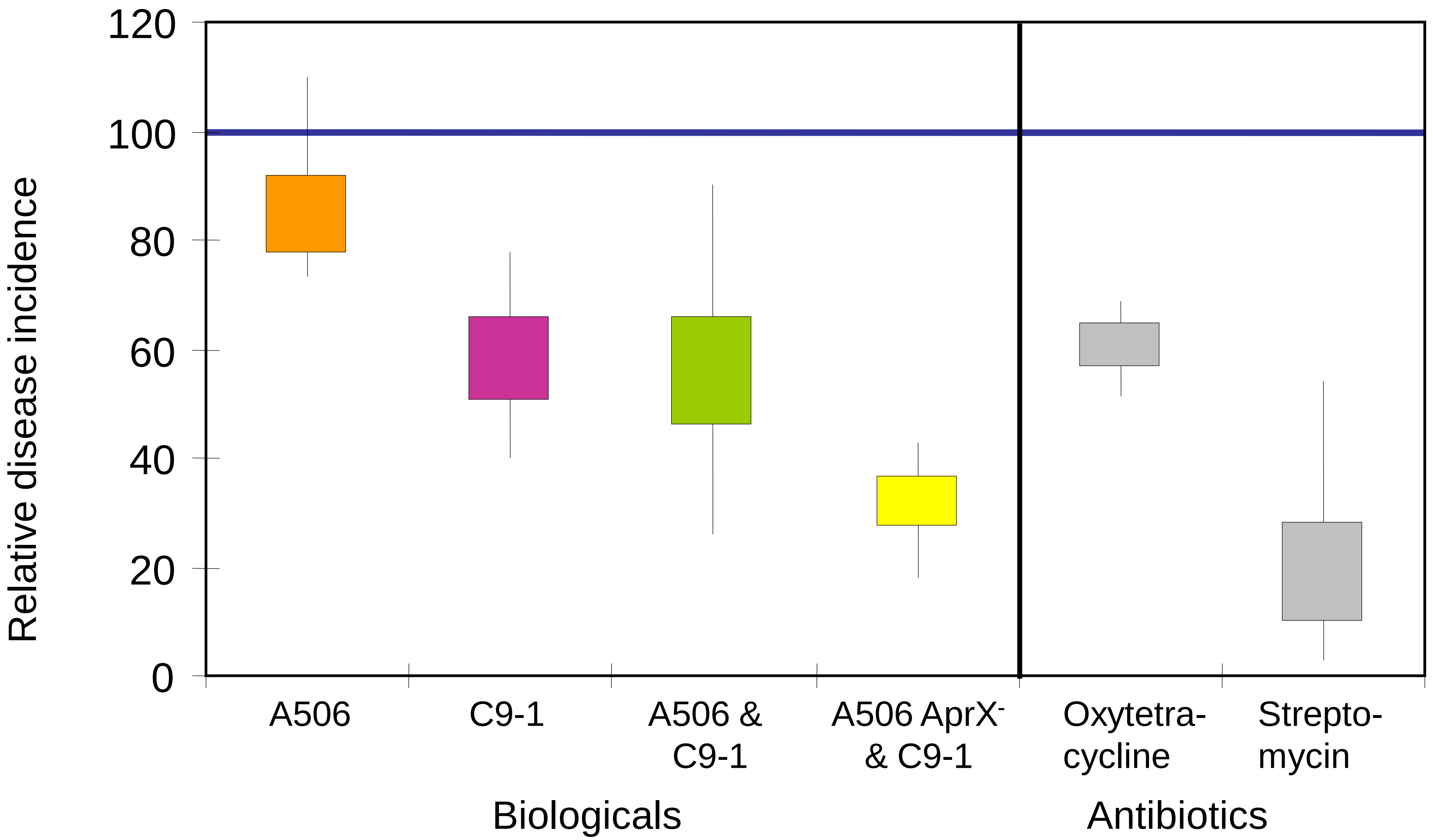
Anderson, Stockwell, & Loper. 2004. Phytopathology 94:1228-1234.

# Disease control with *Pantoea agglomerans* Eh252 & *Pseudomonas fluorescens* A506 *aprX*::Tn*5*




Stockwell et al 2011 Phytopathology

# Disease control with *Pantoea vagans* C9-1 & *Pseudomonas fluorescens* A506 *aprX*::Tn*5*




Stockwell et al 2010 Phytopathology

# Why use mixtures of antagonists for control of fire blight?

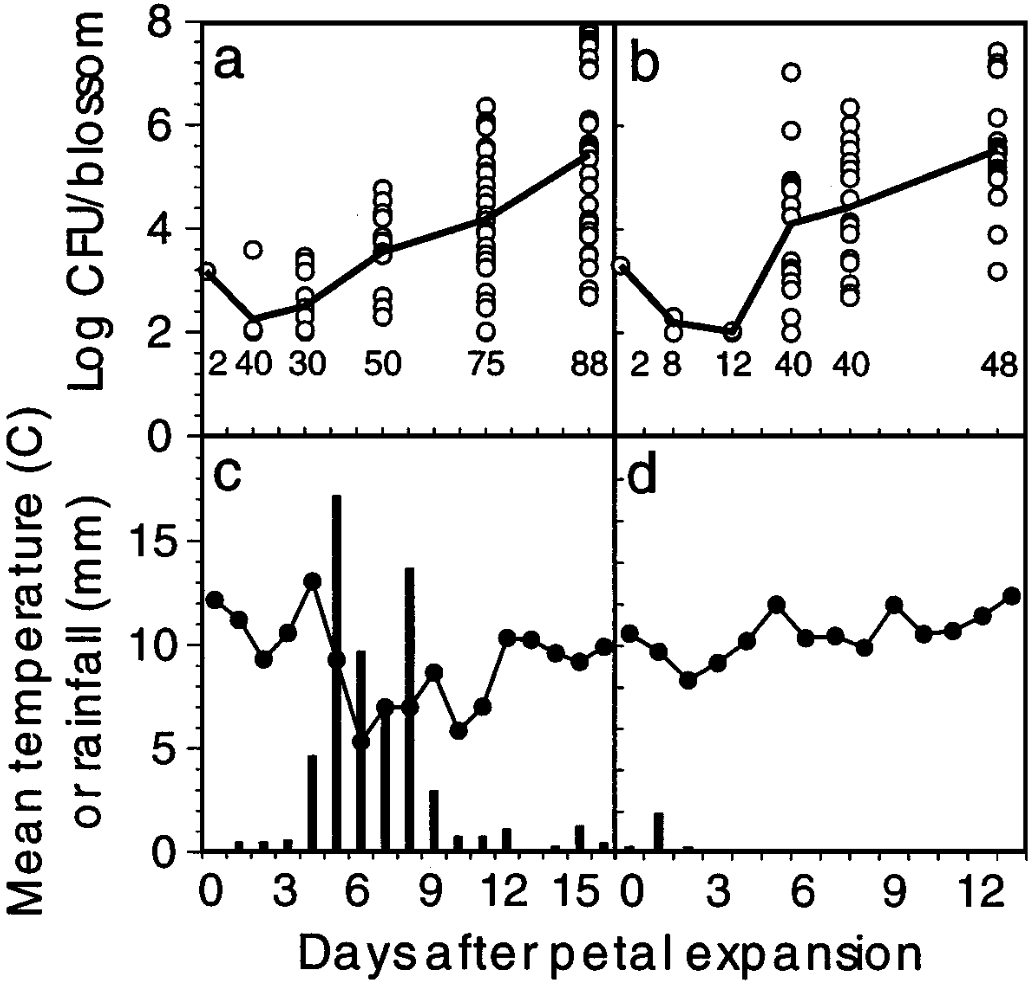
- Greater population sizes with mixed inocula than single strain inoculants.

- Better disease control with compatible mixtures.

- Less variation in efficacy with compatible mixtures compared to single strains.

- Many pseudomonads produce extracellular metalloproteases.
    - We suspect that indigenous pseudomonads may reduce disease control by *Pantoea* spp., thereby contributing to variable efficacy.

# Colonization of pear flowers by orchard bacteria




-Few bacteria are recovered from newly opened flowers.
-Applying bacterial antagonists reduced the incidence of detection of indigenous bacteria by petal fall
-Populations of indigenous bacteria were reduced 1000-fold on flowers colonized by biocontrol agents

Stockwell et al. 1999. Phytopathology 89:1162-1168.

# Why use mixtures of antagonists for control of fire blight?

•Greater population sizes with mixed inocula.
•Better disease control with compatible mixtures.
•Less variation in efficacy with compatible mixtures compared to single strain inoculants.

•Many pseudomonads produce extracellular metalloproteases and mixtures of biocontrol agents reduce their populations.

•Conservation of antibiotic sensitivity of the pathogen. Mixtures of biocontrol agents also reduce populations of indigenous bacteria carrying antibiotic resistance genes, thereby reducing the probability of *Erwinia amylovora* acquiring genes for resistance to agricultural antibiotics.

# Summary

Biological control of crown gall and fire blight represent two successes. Products are commercially available and are used by growers.

Inter-microbial interactions could have affected sustainable control with these antagonists:

1) Biological control of crown gall is an example where interactions between pathogens and the antagonist may lead to loss of efficacy.

   -Genetically modified derivative of K84 unable to transfer its plasmid to the pathogen mitigated this problem. Product is called “NOGALL.”

2) Biological control of fire blight is an example where interactions between antagonists (or antagonist and indigenous microbes) can reduce efficacy.

   -Extracellular protease deficient derivate of A506 reduced variation in disease control with mixtures of antagonists, future commercial use uncertain.

Use of molecular methods may reveal other impacts of intermicrobial interactions on sustainable, efficacious biological control. Reducing sources of variation in biocontrol effectiveness likely will lead to more adoption of microbial-based pest management strategies by growers.

Thank you for your attention...

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